DOI:
10.1177/0333102418756863

Document Version
Peer reviewed version

The role of the brainstem in migraine: Potential brainstem effects of CGRP and CGRP receptor activation in animal models

Philip Robert Holland*, Chonlawan Saengjaroentham and Marta Vila-Pueyo

Headache Group, Basic and Clinical Neuroscience, Institute of Psychiatry, Psychology and Neuroscience, King's College London. UK.

*Corresponding author:

Dr Philip R Holland

Department of Basic and Clinical Neuroscience, Institute of Psychiatry, Psychology & Neuroscience, King's College London, 125 Coldharbour Lane, London, UK

Telephone: +44 02078484222

Email: Philip.holland@kcl.ac.uk

Abstract

Background: Migraine is a severe debilitating disorder of the brain that is ranked as the 6th most disabling disorder globally, with respect to disability adjusted life years and there remains a significant unmet demand for an improved understanding of its underlying mechanisms. In conjunction with perturbed sensory processing, migraine sufferers often present with diverse neurological manifestations (premonitory symptoms) that highlight potential brainstem involvement. Thus, as the field moves away from the view of migraine as a consequence of purely vasodilation to a greater understanding of migraine as a complex brain disorder, it is critical to consider the underlying physiology and pharmacology of key neural networks likely involved.

Discussion: The current review will therefore focus on the available evidence for the brainstem as a key regulator of migraine biology and associated symptoms. We will further discuss the potential role of CGRP in the brainstem and its modulation for migraine therapy, given the emergence of targeted CGRP small molecule and monoclonal antibody therapies.

Conclusion: The brainstem forms a functional unit with several hypothalamic nuclei that are capable of modulating diverse functions including migraine-relevant trigeminal pain processing, appetite and arousal regulatory networks. As such, the brainstem has emerged as a key regulator of migraine and is appropriately considered as a potential therapeutic target. While currently available CGRP targeted therapies have limited blood brain barrier penetrability, the expression of CGRP and its receptors in several key brainstem nuclei and the demonstration of brainstem effects of CGRP modulation highlight the significant potential for the development of CNS penetrant molecules.

Keywords: Migraine, Brainstem, CGRP.

Introduction

Migraine is a severe debilitating disorder of the brain that has troubled humans for centuries (1). Despite representing the 6th most disabling disorder globally (2), and the most disabling neurological condition with respect to disability adjusted life years, there remains a significant unmet demand for an improved understanding of the underlying disease mechanisms. Several theories have been proposed for migraine-related pain, including vasodilation (3, 4), neurogenic inflammation (5) and activation of the trigeminal pain processing primary afferent nociceptors that innervate the intra- and extra-cranial structures of the head (3, 6). In conjunction with the developing clinical picture, whereby migraine is considered a multiphasic disorder, current theories of migraine propose a complex neural origin with subsequent vascular involvement (6, 7).

While head pain is a seminal characteristic of migraine (8) patients often present with several associated neurological symptoms. These include perturbed sensory responses to light (photophobia), sound (phonophobia), smell (osmophobia) and touch (allodynia) (9). In addition to this abnormal sensory processing, cognitive, emotional and motor abnormalities are commonly reported by migraine patients that may be confounded by several comorbid conditions including insomnia (10) and depression (11). Given the diversity of symptoms, migraine likely involves the interaction of several neural networks that regulate these diverse functions (e.g. nociception, arousal and appetite) (7). As evidenced by the presence of several common premonitory (prodromal) symptoms (12) that include disrupted homeostatic mechanisms regulating sleep/wake and appetite regulation (e.g. fatigue, yawning, loss of appetite). Thus, as the migraine field moves away from the view of migraine as a consequence purely of vasodilation (13-15) to a greater understanding of migraine as a brain disorder (1, 6, 7), it is critical to consider the underlying pathways known to be involved in the diverse symptoms of migraine. In doing so, it is further

important to consider if anti-migraine therapeutic agents, such as targeted calcitonin gene-related peptide (CGRP) modulation, may in part act via these networks? As such, the current review will focus on the available evidence for the brainstem as a key regulator of migraine biology. We will further discuss the potential role of CGRP in the brainstem and its modulation for migraine therapy, given the emergence of targeted CGRP small molecule and monoclonal antibody therapies.

Migraine-related pain processing and the brainstem

Undoubtedly, one of the most salient features of migraine is the severe debilitating often unilateral pulsating head pain that is characteristic of the condition. This is borne from the activation, or perceived activation, of the pain processing trigeminovascular system that processes sensory information from the intra- and extra-cranial structures of the head and face (Figure 1). The exact role of trigeminovascular activation is still debated. Does an underlying CNS disruption result in the diverse symptoms associated with migraine, whilst driving aberrant trigeminovascular activation, or is trigeminovascular activation sufficient to trigger an attack and drive the diverse symptoms? Irrespective of the sequence of events, it is clear that the pain processing trigeminovascular system and its central projections represent a key interface between migraine-related pain and its associated non-pain features (Figure 2). As such, activation of the trigeminovascular system has been consistently used as a preclinical model of migraine-related pain processing (nociception). Primary sensory afferents arising in the trigeminal ganglion (TG) project peripherally to the intra- and extracranial structures, including the pial and dural blood vessels (3, 16, 17), as well as projecting centrally to the trigeminal nucleus caudalis and its cervical boundaries (18-23), giving rise to the trigeminocervical complex (TCC) (Figure 1). From the TCC, second order neurons ascend in the trigemino- and quintothalamic tracts where they primarily

synapse on ventral posteromedial nucleus of the thalamus (24-28), before being widely distributed throughout the cortex for the integration of sensory, affective and cognitive aspects of pain. Critically, these ascending trigeminothalamic projections form additional connections with areas of the brainstem including the periaqueductal grey (PAG), rostral ventromedial medulla (RVM), nucleus raphe magnus (MRN) and the locus coeruleus (LC) (24, 29), as well as to various hypothalamic nuclei (30-34).

The importance of the trigeminal-brainstem interactions is highlighted in several neuroimaging studies that demonstrate brainstem activation during spontaneous (35) and triggered attacks (36). Whilst this activation was originally suggested to be in response to the developing headache, recent observations have highlighted that brainstem activation occurs during the earliest premonitory phases (37) prior to the headache. This is further supported by the demonstration of altered brainstem functional connectivity (hypothalamus-PAG) in a temporally defined phase prior to the onset of spontaneous migraine attacks (38). Despite the clear evidence for brainstem involvement, the specific brainstem structures involved are still debated due to the limited resolution of neuroimaging approaches. Further, it is not clear if this altered brainstem activation is occurring independent of an increasing activation of the trigeminovascular system, as it has been shown that the TCC increases its responsiveness to noxious intranasal stimuli as the next migraine attack approaches (39). The brainstem may act as a component of a homeostatic regulatory network that monitors and reacts to increasing trigeminovascular activity in response to endogenous and exogenous signals that can predispose an individual to attacks.

In addition to receiving direct ascending TCC projections and being activated in response to nociceptive durovascular stimulation in animal models (19, 40-44), these brainstem structures, that likely include the PAG, LC, RVM and MRN are intrinsically linked throughout the

trigeminovascular system, including to higher order structures such as the hypothalamus and thalamus (Figure 1) (1). This diverse network of brainstem nuclei is ideally positioned to integrate trigeminal mediated signals with neural networks regulating migraine-associated symptoms (Figure 2) and represents a potential target for neuromodulatory and pharmacological approaches for migraine.

The Brainstem and homeostatic regulation

The hypothalamus has emerged as a key regulator of homeostatic mechanisms (45), sensing intrinsic and extrinsic signals while orchestrating appropriate behavioural responses. While the hypothalamus resides within the diencephalon, it is intrinsically linked with several brainstem nuclei forming a functional homeostatic regulatory network. As such, the brainstem plays a significant role in multiple migraine-relevant biological functions (as detailed in Figure 2). For example, they play a key role in the integration of local and circulating factors that convey information regarding energy balance acting to regulate appetite and energy expenditure (46). Vagal afferents from the gastrointestinal tract signal via the nucleus of the solitary tract (NTS) and the area postrema (AP; also plays a role in nausea (47)) to the hypothalamic nuclei regulating appetite. Critically, this appetite-regulating pathway, including the AP and NTS has been shown to express functional CGRP receptor components (Figure 3 and Table 1), while lesions in the area of the AP/NTS abolishes CGRP induced anorectic effects (48). It is noteworthy that the AP is devoid of the BBB (49), suggesting it is amenable to peripherally administered CGRP therapies; however, the functional relevance of this localized BBB permeability remains to be determined (see section on CGRP and the BBB).

Further, several brainstem nuclei regulate arousal levels (Figure 2) and therefore have been linked to the presence of abnormal fatigue-like symptoms during migraine (12, 50). The majority of these structures, including the PAG and LC, receive wake-promoting projections from the hypothalamus (for review see (51)), contain CGRP fibers/cell bodies and express CGRP receptor components (52-55) (see Figure 3 and Table 1 for detailed distribution). Given their established roles in the modulation of trigeminovascular activity and cerebral blood flow (56-59) they likely form a key interface between migraine and its association with sleep/wake disturbances (60). As such, the brainstem is ideally positioned to regulate several biological functions related to migraine, including appetite and fatigue (arousal dysregulation), is abnormally active during the earliest attack phases and has been proposed to play a role in attack initiation.

Table 1. CGRP receptor expression, CGRP binding patterns and CGRP expression in selected brainstem nuclei.

The data is further presented in figure form in figure 3. AP; area postrema, CB; cell bodies, CLR; calcitonin receptor-like receptor, DMV; dorsal motor nucleus of the vagal, DR; dorsal raphe, F; Fibers, LC; locus coeruleus, MK-3207; CGRP receptor antagonist, Mod; moderate, MRN, nucleus raphe magnus, NTS; nucleus of the solitary tract, PAG; periaqueductal gray, RAMP1: receptor activity modifying protein 1, RCP, receptor component protein, SuS; superior salivatory nucleus, TCC; trigeminocervical complex, VN; vestibular nuclei

	CGRP receptors components/CGRP Binding	CGRP Expression	Species	Ref
AP	CLR & RAMP1 (Protein & mRNA); MK-3207 Binding	N/A	Primate	(53)
	N/A	F (High)	Alpaca	(61)
DMV	RAMP1 (F)	CB	Rat	(62)
	RCP (Mod CB)	CB (Mod)	Rat	(54)
	N/A	F (Low); CB (High)	Alpaca	(61)
	CLR & RAMP1 (Protein & mRNA); MK-3207 Binding	N/A	Primate	(53)
	GRP Binding (High)	N/A	Human/Rat	(63)
DR	RAMP1 (mRNA); CLR & RAMP1 (Protein); MK-3207 Binding	N/A	Primate	(53)
	CGRP Binding (Mod)	N/A	Human	(63)
	N/A	F (Low); CB (High)	Alpaca	(61)
	RCP (Mod Cell Bodies)	Axons & CB (Low)	Rat	(54)
LC	N/A	CB (80%); F (Low)	Human	(55)
	RAMP1 & CLR (F & CB)	CB	Rat	(62)
	N/A	F (Mod)	Alpaca	(61)
	RCP (Mod CB)	CB (Mod)	Rat	(54)
	CGRP Binding (High)	N/A	Human/Rat	(63)
MRN	RAMP1 & CLR (F & CB)	CB	Rat	(62)
NTS	Absent	F & CB	Rat	(62)
	N/A	F (High)	Alpaca	(61)
	RCP (Mod CB)	Axons (Mod)	Rat	(54)
	CGRP Binding (High)	N/A	Human/Rat	(63)
PAG	CLR & RAMP1 (Protein & mRNA); MK-3207 Binding	N/A	Primate	(53)
	RCP (Mod CB)	Axons (Low)	Rat	(54)
	CLR & RAMP1 (Cell Bodies)	N/A	Rat	(64)
	CLR & RAMP1 mRNA (High); RCP mRNA (Mod)	*CALCA* mRNA (Low)	Rat	(65)
	N/A	F & CB (Low)	Alpaca	(61)
	CGRP Binding (Moderate)	N/A	Rat	(66)
	CGRP Binding (High)	N/A	Human/Rat	(63)
SuS	N/A	Fibers	Rat	(67)
TCC	CLR & RAMP1 (Protein & mRNA); MK-3207 Binding	N/A	Primate	(53)
	RCP (Mod CB)	Axons (Mod)	Rat	(54)
	CLR/RAMP1	Present (not defined)	Rat	(62)
	CLR mRNA (High); RAMP1 & RCP mRNA (Mod)	*CALCA* mRNA (Mod)	Rat	(65)
	CGRP Binding (High)	N/A	Human	(63)
	CLR & RAMP1 (CB)	N/A	Rat	(64)
	CGRP Binding (Mod)	N/A	Rat	(66)
VN	N/A	F & CB	Rat	(68)
	N/A	CB	Rat	(69)
	CGRP Binding (Mod)	N/A	Rat	(66)
	CGRP Binding (High/Mod)	N/A	Human/Rat	(63)

Calcitonin Gene-Related Peptide

CGRP belongs to the calcitonin family and is synthesized from two genes; the *CALCA* gene gives rise to calcitonin or αCGRP and the distinct *CALCB* gene gives rise to βCGRP (57). The two isoforms are similar in homology and biological function, however αCGRP is classically considered to predominate in the peripheral and central nervous system, whereas βCGRP predominates in the enteric nervous system (70), as such we will focus on αCGRP (described as CGRP from this point onward). Following the synthesis of CGRP, a process that remains poorly understood, the neuropeptide is stored in large, dense-core vesicles within nerve terminals (71).

As discussed previously, CGRP is expressed in sensory afferents innervating the cranial vasculature (72) where it can act as a potent vasodilator. However, its widespread distribution throughout the trigeminal ganglion and afferents, brainstem, diencephalic and particularly the cerebellum, highlights its diverse role in neurotransmission (73). The CGRP receptor consists of three key components: (i) the calcitonin receptor-like receptor (CLR), (ii) a receptor activity modifying protein (RAMP) and (iii) an additional receptor component protein (RCP). The CLR belongs to the same "secretin-like" family of G protein-coupled receptors as those for calcitonin, vasoactive intestinal peptide and pituitary adenylate cyclase-activating peptide and when expressed independently it is unresponsive to CGRP. CLR requires a RAMP to regulate its functional specificity and membrane translocation (73). The association of CLR with RAMP1 denotes the established CGRP receptor, CLR and RAMP2 results in the adrenomedullin receptor (AM_1 receptor) and CLR and RAMP3 results in an additional adrenomedullin receptor (AM_2 receptor). While the primary receptor for CGRP is considered to be the CLR/RAMP1 complex, it is known that *in vitro* CGRP shows partial affinity for the CLR/RAMP3 AM_2 receptor (74) and for a recently described third potential CGRP receptor resulting from the combination of the

calcitonin receptor and RAMP1 (75). Given the widespread distribution of CLR, it is perceived that individual RAMPs are responsible for receptor tissue (76) and species specificity (77).

The above combination of receptor proteins and RAMPs constitutes a functional receptor; however, the presence of a third RCP is essential for optimal function (78). The RCP is a small hydrophilic membrane-associated protein that enhances the signal transduction of both CGRP and adrenomedullin receptors, suggesting that it may represent a novel therapeutic target for the inhibition of all identified potential CGRP receptors.

Targeted CGRP Therapies and the Blood-Brain Barrier (BBB)

Currently targeted CGRP therapies are focused on either acute CGRP receptor antagonism (gepants) or prophylaxis with monoclonal antibodies that bind to the CGRP peptide or its receptor (umabs). Targeted antagonism of the CGRP receptor has demonstrated significant clinical promise (79). The initial doses required for clinical efficacy raised the possibility that the small molecules may have to penetrate the BBB to exert their effects (80). This was supported by previous animal research demonstrating that olcegepant inhibited capsaicin-induced sensitization in the TCC with little impact on the TG (81). Despite this initial focus on potential central effects, telcagepant has subsequently been shown to bind to the TG of rhesus monkeys, while failing to displace central binding of the radiolabeled CGRP PET ligand MK-4232 (82), suggesting limited CNS bioavailability in humans. This proposed peripheral site of action is further supported by the demonstration of a clear clinical efficacy for umabs (83), whose large molecular weight largely preclude BBB penetration, with only 0.1% of systemic values estimated to gain access to the CNS. In view of this relative lack of BBB permeability, it has alternatively been proposed that the BBB

is compromised during migraine attacks and, as such, the precise location of action of targeted CGRP therapies and existing migraine therapies remains a consistently debated topic. It should, however, be noted that studies utilizing radiolabeled dihydroergotamine found no evidence for BBB disruption during migraine attacks (84).

Independent of the potential disruption of the BBB during migraine, it is clear that specific brain areas lack a functional BBB and, as such, these sites offer a potential conduit for larger molecules to enter the CNS and/or facilitate signaling via widespread CNS projections. Specific areas include the circumventricular organs, meningeal arteries, pineal gland, preoptic recess and endothelium of the choroid plexus. The circumventricular organs are particularly interesting (85), these include both sensory (subfornical organ (SFO), vascular organ of the lamina terminalis (OVLT) and the AP) and secretory organs (neurohypophysis, median eminence and the pineal gland). The SFO, OVLT and AP are reciprocally interconnected with each other and with several hypothalamic and brainstem nuclei that play important roles in migraine pathophysiology. The SFO receives significant afferent input from the lateral and anterior hypothalamus, as well as the paraventricular nucleus, while its efferent projections signal to the suprachiasmatic nucleus and paraventricular nucleus. The OVLT is additionally innervated by direct projections from the dorsomedial, ventromedial and preoptic hypothalamic nuclei, as well as from the PAG and LC. It sends efferent fibers to the paraventricular, supraoptic, preoptic and lateral hypothalamic nuclei, as well as to the PAG and LC. In terms of direct brainstem access, the AP forms part of the vagal complex in conjunction with the NTS and the DMV. It is reciprocally interconnected with the other circumventricular organs in addition to the NTS and RVM, and receives direct afferent projections from the paraventricular nucleus. The AP has been shown to express CGRP receptors (Figure 3 and Table 1)(52, 53) and together with other circumventricular organs is involved in the regulation

of feeding/energy homeostasis and nausea. The functional importance for these BBB openings in relation to migraine therapy remain to be explored.

In light of this limited CNS penetrability, the concept of exploring brainstem effects of current CGRP therapies appears on the surface a fruitless endeavor. Yet, as will be discussed below, there is clear evidence for potential CNS mechanisms of CGRP modulation. This is in agreement with the demonstration of brainstem effects of triptans (58), despite variable BBB penetrability that has ultimately led to the development of a novel class of CNS penetrant, centrally acting 5-HT_{1F} receptor agonists (ditans) (86). Taking this into consideration, it is reasonable to conclude that the development of CNS penetrant CGRP targeted therapies or novel BBB transporters remains a valid target for migraine therapy.

CGRP expression in the brainstem

As detailed above, αCGRP (herein denoted as CGRP) predominates in the CNS (70) and its expression has been widely mapped throughout the brainstem ((52-55), which the following section is based upon, see Figure 3 and Table 1 for a detailed breakdown of expression). Given the widespread expression of CGRP and its receptors, it has been linked to a number of functions ranging from nociception (87) to vestibular regulation and motion sickness (68), all of which have been linked to migraine. CGRP expression is largely conserved across species, including human, monkeys, cats and rodent tissue, and, as such, expression data is discussed independent of species except where notable differences have been identified. Small and medium size CGRP-expressing trigeminal primary afferents arising from the TG synapse within the superficial laminae of the TCC, where they synapse on ascending trigeminothalamic projection neurons (88). Clinical and

preclinical data consistently highlight the importance of the TCC as a key transitional interface in the processing of trigeminovascular nociception, that additionally receives descending modulatory inputs from several brainstem and higher order structures (Figure 1). The highest density of CGRP immunoreactivity in the brainstem has been reported in the LC (55, 89), with moderate expression observed in the PAG. Additional expression has been observed throughout several brainstem structures, including the dorsal raphe nuclei (DR), spinal trigeminal nuclei and vestibular nuclei (VN). Given the complexity of the CGRP receptor structure, a variety of approaches have attempted to visualize CGRP receptor distribution in the brainstem. The RCP component demonstrates high expression levels in the PAG and DR nuclei of rats, with moderate expression in the ventral tegmental area and LC; while CLR is observed in the human brainstem nuclei including the NTS and AP. In support of the widespread distribution of CGRP receptors, a recent study utilizing [^{11}C]MK-4232 as a selective marker for the CLR/RAMP1 complex demonstrated widespread moderate distribution in the human and primate brainstems, although specific brainstem nuclei were not defined. (82).

Brainstem effects of CGRP and CGRP receptor modulation

CGRP is widely distributed in the brainstem, yet relatively little is known regarding its specific function. Of particular importance to migraine is the predominance of CGRP in small to medium trigeminal primary afferents that synapse in the dorsal horn of the TCC. CGRP release at these central synapses is suggested to increase nociceptive transmission. Local delivery of CGRP onto dural nociceptive-responsive TCC neurons in the cat resulted in excitation of over 40% of neurons tested. Further, CGRP antagonism with the truncated form of CGRP ($CGRP_{8-37}$) or the small molecule antagonist olcegepant (BIBN4096BS) inhibited the response of the majority of neurons

to local glutamate, suggesting a post-synaptic mechanism of action (90). In agreement, *ex vivo* studies on medullary brain slices demonstrated increased CGRP release in response to potassium chloride and capsaicin that was inhibited by naratriptan. These results highlight the importance of CGRP release from central terminals in the TCC for trigeminal nociception and suggest that TCC CGRP modulation may be a potential mechanism of action for the triptans (91).

As noted, the highest expression levels of CGRP in the brainstem are found in the noradrenergic LC (55), an area involved in the regulation of arousal (92), autonomic (93), stress (94), nociceptive functions (95) and cerebral blood flow (59, 96, 97) (Figure 2). Preclinically, the LC is responsive to activation of the trigeminovascular system (98) and known clinical migraine triggers, including nitroglycerin, induce LC activation (99); while its stimulation in the cat impacts cerebral blood flow inducing hypoperfusion (59, 96, 97) reminiscent of the functional hyperemia observed during cortical spreading depression. The LC is further activated by CTR-stimulating peptide 1, suggesting that it is sensitive to circulating CGRP levels that may act to regulate energy homeostasis (100). Inhibition of CGRP receptors with olcegepant *in vitro* in organotypic brain slice cultures containing the LC increases noradrenaline release, suggesting a possible action of CGRP targeted therapies on intrinsic CGRP signaling in the LC, which could impact on migraine pathophysiology (101). While the exact functions of the LC remain to be fully elucidated, its role as a major arousal promoting network that demonstrates clear diurnal activation patterns (102), has highlighted its potential as a regulator of migraine biology. This link has been recently strengthened by the demonstration of altered trigeminovascular dural nociceptive evoked responses following lesioning of the LC (103), whereby acute lesioning or chronic ablation of the LC reduced dural-nociceptive evoked TCC responses.

The PAG is perhaps the most prominent brainstem nuclei in migraine research since Weiller et al. (35) demonstrated its activation during a spontaneous migraine attack, with subsequent imaging studies highlighting abnormal functional activation and network connectivity during the premonitory phase (37, 38). Preclinically, this is in agreement with abnormal functional connectivity states following repetitive dural inflammatory soup application (104), suggesting that abnormal PAG signaling may be an important regulator of/response to recurrent attacks. Electrical or chemical activation of the ventrolateral subunit of the PAG (vlPAG) inhibits durovascular evoked trigeminal nociceptive processing in the TCC (105-107), including local application of a triptan (58). It has further been demonstrated that orally administered rizatriptan, that is thought to have a degree of central anti-nociceptive effects (108), reduces nitroglycerin-induced elevation of CGRP in the PAG (109), thus highlighting the potential of targeting such brainstem nuclei in migraine therapy. While the PAG is protected by the BBB, direct administration of CGRP into the PAG has been shown to potentiate trigeminovascular nociception at the level of the TCC, and both $CGRP_{8-37}$ and olcegepant inhibit TCC dural-nociceptive evoked responses in the rat (64). It must be noted, however, that direct administration of CGRP into the MRN of rats demonstrated an anti-nociceptive effect on hind-paw thermal and mechanical stimuli (110). As such, there is evidence for complex pro- and anti-nociceptive effects of CGRP signaling in brainstem nuclei that may be dependent on the specific nuclei or the pain modality being tested.

The RVM is a key output relay of the PAG involved in the indirect modulation of pain signaling. It has reciprocal connections with the PAG and TCC (111-113) and is known to contain both anti-nociceptive off and pro-nociceptive on cells (113) that can regulate trigeminovascular nociception. For example, repetitive application of an inflammatory soup to the dura mater of rats results in heightened mechanosensation via an action on RVM mediated descending facilitation (41). This

pathway has further been implicated in the regulation of homeostatic mechanisms as on-cells are state dependently active during wakefulness; however, they fall silent during feeding. Off-cells show an opposite response to feeding in that they are constituently activated during feeding, suggesting a role in the integration of energy homeostasis (114, 115). In the context of CGRP, there is little evidence for CGRP/CGRP receptor expression within the RVM (116); however, it is likely that the RVM acts downstream of other brainstem and hypothalamic nuclei including the PAG that do show responsiveness to CGRP signaling (64).

Patients with migraine often report vertigo (117), which is regulated by the vestibular nuclei in the brainstem. CGRP is expressed throughout the various vestibular nuclei (118-120) (Figure 3 and Table 1), with preclinical studies identifying increased CGRP expression in a rotational model of motion sickness (121). Rats exposed to repeated bouts of rotary stimulation had an increased number of CGRP expressing neurons throughout the vestibular nuclei, including the vestibular efferent nucleus at the level of the facial nerve genu. This is in agreement with a reduced vestibule-ocular reflex in CGRP null mice (122) highlighting the potential role of elevated CGRP signaling as an underlying mechanism for the association between migraine and vertigo (123).

Conclusion

The brainstem forms a key functional unit with key hypothalamic nuclei that is capable of modulating diverse functions including migraine-relevant trigeminal pain processing, appetite and arousal regulatory networks. While the resolution of human neuroimaging precludes the identification of specific nuclei, it does highlight the abnormal activation of a brainstem nuclear complex during the earliest premonitory phase that continues into the headache phase and remains

after triptan-induced pain relief (35). As such, the brainstem has emerged as a key regulator of migraine biology and is appropriately considered as a potential therapeutic target. CGRP and its receptors are expressed throughout the brainstem (detailed in Figure 3 and Table 1) where their modulation has been shown to impact on trigeminal mediated pain and associated migraine-related functions. Current evidence suggests that the majority of CGRP targeted therapies, including monoclonal antibodies targeting CGRP or its receptor, do no cross the BBB in any significant quantity. Despite this lack of BBB penetration (notwithstanding localized access at areas such as the AP), there is sufficient clinical and experimental data to suggest that targeting central mechanisms including via brainstem nuclei may offer additional therapeutic benefits.

Article Highlights

- The brainstem is a key region involved in the regulation of homeostatic mechanisms that play a prominent role in migraine including fatigue (arousal dysfunctions) and appetite.
- Several brainstem nuclei contain CGRP and its receptor and local application of CGRP agonists and antagonists can modulate trigeminovascular nociception.
- Modulation of brainstem CGRP signaling remains a valid target for the development of brain penetrant CGRP targeted therapies.

Review Process

We searched PubMed, Medline and Web of Science with the search terms “migraine”, “brainstem”, “CGRP”, “CGRP receptor”, “periaqueductal gray”, “locus coeruleus”, “rostral ventromedial medulla”, “trigeminal” and combinations therein. We reviewed bibliographies of relevant articles and identified articles through additional author searches to include mostly preclinical articles and occasional congress proceedings/abstracts published in English. The final reference list was then selected based on the authors perception of the relevance of the article to the scope of this review.

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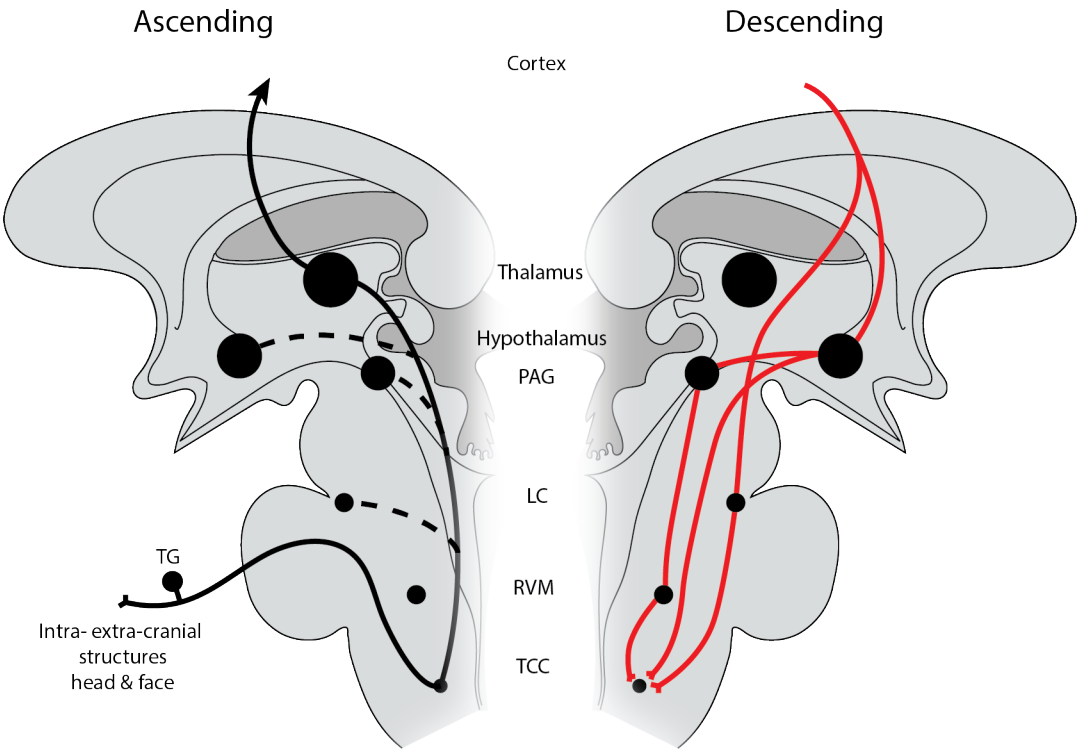

Figure 1: Ascending and descending projections via the brainstem in migraine.

The trigeminal ganglion (TG) gives rise to the pseudo-unipolar trigeminal primary afferents which synapse on the intra- and extra-cranial structures (blood vessels and dura mater) as well as the spinal cord trigeminocervical complex (TCC). Second-order neurons from the TCC ascend in the trigeminothalamic tract synapsing on third-order thalamocortical neurons before passing to the cortex. Direct and indirect ascending projections additionally exist to key brainstem and diencephalic nuclei including the locus coeruleus (LC), periaqueductal grey (PAG) and hypothalamus. The TCC is under the regulation of direct and indirect descending modulatory pathways. Direct and indirect projections (via the hypothalamus) exist from the cortex, whilst key brainstem nuclei including the PAG, LC and rostral ventromedial medulla (RVM) provide modulatory pathways that arise within the nuclei or are under top down regulation. This complex network of descending modulatory circuits potently regulates the TCC providing pro- and anti-nociceptive drive.

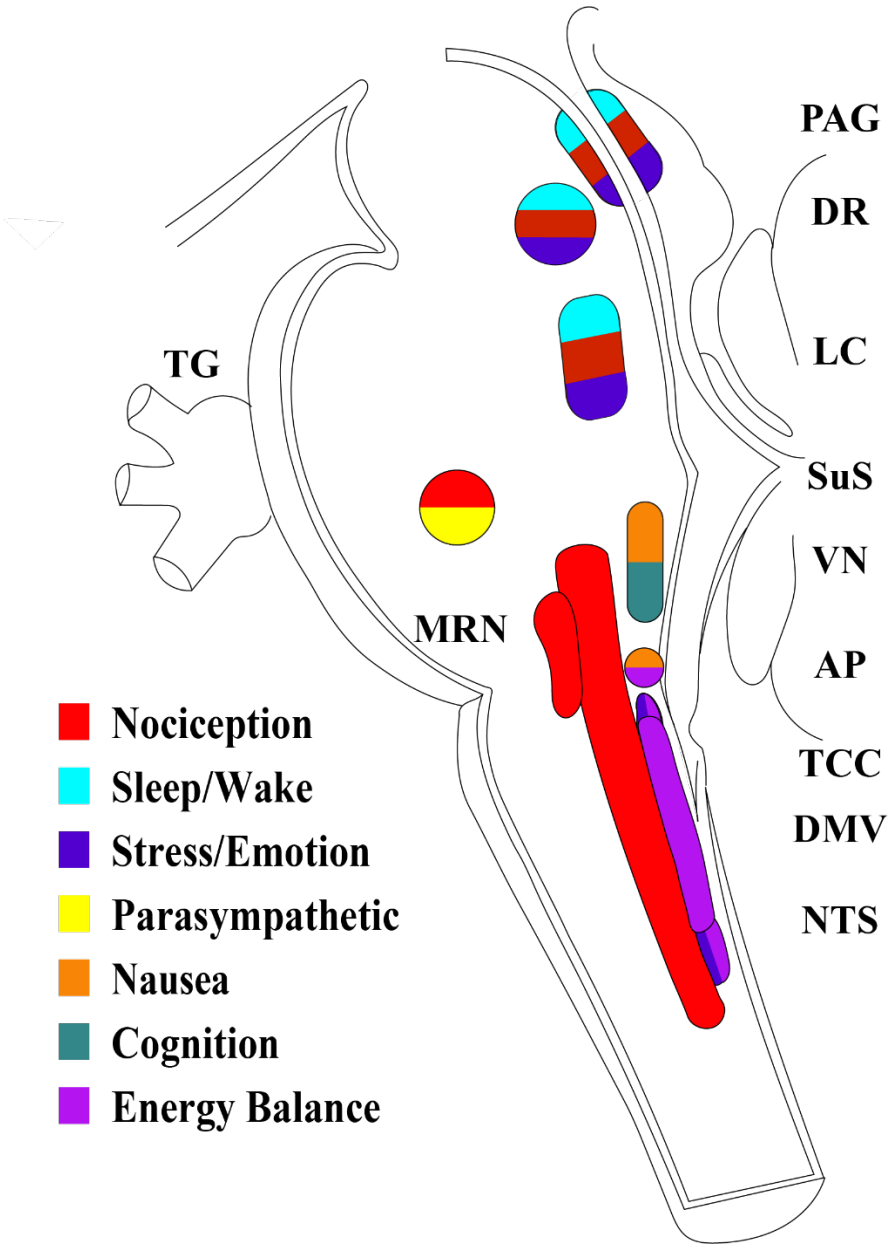

Figure 2: The functional importance of selected brainstem nuclei with respect to migraine-associated symptoms.

AP; area postrema, DMV; dorsal motor nucleus of the vagal, DR; dorsal raphe, LC; locus coeruleus, MRN, nucleus raphe magnus, NTS; nucleus of the solitary tract, PAG; periaqueductal gray, SPG; sphenopalatine ganglion, SuS; superior salivatory nucleus, TCC; trigeminocervical complex, TG; trigeminal ganglion, VN; vestibular nuclei.

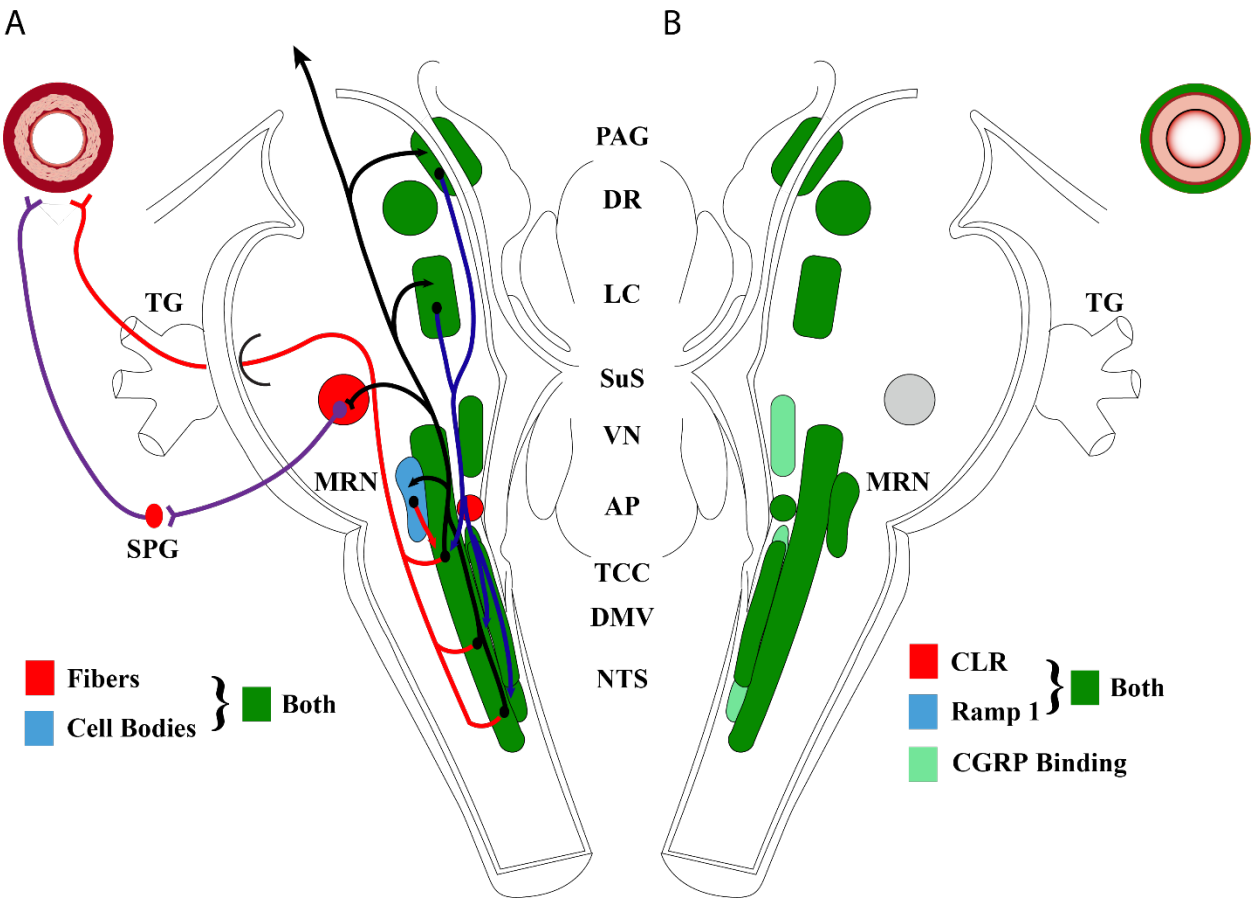

Figure 3: Overview of CGRP expression in selected brainstem nuclei.

(A) The expression of CGRP fibers (red), cell bodies (soma, blue) or both (green) in selected brainstem nuclei are shown. (B) The expression of calcitonin receptor-like receptor (CLR, red), receptor activity modifying protein 1 (RAMP 1, blue) or both (green) in selected brainstem nuclei are shown. Where CLR and RAMP 1 expression is unknown, CGRP receptor binding has been used to highlight the presence of functional CGRP receptors (light green). The above information is taken from the references and data presented in Table 1. AP; area postrema, DMV; dorsal motor nucleus of the vagal, DR; dorsal raphe, LC; locus coeruleus, MRN, nucleus raphe magnus, NTS; nucleus of the solitary tract, PAG; periaqueductal gray, SPG; sphenopalatine ganglion, SuS; superior salivatory nucleus, TCC; trigeminocervical complex, TG; trigeminal ganglion, VN; vestibular nuclei.